United States Department of Agriculture
Agricultural Marketing Service, Science & Technology
Microbiological Data Program

SOP No.: MDP-MTH-06		Page 1 of 7
Title: *Escherichia coli* O157 Immunomagnetic Separation (IMS) Method and Confirmation		
Revision: Original	Replaces: NA	Effective: 04/15/04

1. Purpose:

This Standard Operating Procedure (SOP) is used after completion of SOP MDP-MTH-05 yielding a positive result for *Escherichia coli* (*E. coli*) O157:H7. This document provides standard procedures for capturing *E. coli* O157 cells by immunomagnetic separation, subculturing to CHROMagar O157 plates, and identifying isolates by VITEK. Isolates will be serotyped by reference laboratories.

2. Scope:

This SOP shall be followed by all laboratories conducting microbiological studies for MDP, including support laboratories conducting non-routine activities that may impact the program.

3. Principle:

The immunomagnetic separation (IMS) method offers a means to concentrate target bacteria from mixed cultures by physical separation based on antigen-antibody reaction. Selective capture (concentration) of bacterial cells is achieved by antibodies, specific to the cell surface antigens of the target strain, immobilized on superparamagnetic polysterene micro-beads. After washing the beads to remove non-target organisms, the magnetized beads coated with target bacteria are recovered and processed for isolation and identification by cultural methods.

4. Outline of Procedures:

5. References:

5.1 Enrichment of *E. coli* O157 – Package insert from Dynal Biotech http://www.dynalbiotech.com/ - last accessed 03-10-04.

5.2 USDA/FSIS Microbiology Laboratory Guidebook Chapter 5, Revision 2 Detection, isolation, and identification of *Escherichia coli* O157:H7 and O157:NM (non motile) from meat products, http://www.fsis.usda.gov/ophs/microlab/mlg5.03.pdf, last accessed 03-10-04.

5.3 Weagant, S. D. and A. J. Bound. 2001. Evaluation of techniques for enrichment and isolation of *Escherichia coli* O157:H7 from artificially contaminated sprouts. International Journal of Food Microbiology. 71: 87-92.

5.4 Wu, F. M., L. R. Beuchat, J. G. Wells, L. Slutsker, M. P. Doyle, and B. Swaminathan. 2001. Factors influencing the detection and enumeration of *Escherichia coli* O157:H7 on alfalfa seeds. International Journal of Food Microbiology. 71: 93-99.

5.5 SOP MDP-MTH-05, Detection of *Escherichia coli* O157:H7 in Fresh Produce by BAX system.

5.6 SOP MDP-SHIP-03, Archival of Microbiological Data Program Isolates

5.7 SOP MDP-DATA-01, Microbiological Record Keeping and Results Reporting

6. Specific Procedures:

6.1 Equipment and Materials

6.1.1 Dynal MPC-S (Product No. 120.20), Dynal Biotech

6.1.2 Any shaker, preferably a platform type

6.1.3 Micropipettors to deliver 10-100 µl and sterile disposable filtered micropipette tips

6.1.4 Sterile pipettes and pipet aids

6.1.5 Sterile inoculating swabs, loops, and hockey sticks

6.1.6 1.5 ml microcentrifuge tubes

6.1.7 Incubator 35 ± 2^{o}C

6.1.8 Lab coats, disposable gloves, and protective eye glasses

6.1.9 VITEK (bioMerieux, Inc.) System and User's Manual

6.2 Media, and Reagents

6.2.1 Produce sample culture [modified EC broth with novobiocin (mEC+n)] that tested positive by BAX; refer to SOP MDP-MTH-05

6.2.2 Dynal Biotech's Dynabeads® anti-*E. coli* O157

6.2.3 Wash buffer (PBS Tween): 0.15M NaCl, 0.01M Sodium-Phosphate buffer, pH 7.4, with 0.05% Tween-20; autoclaved at 121°C for 15 minutes. This buffer can also be purchased as liquid or powder. Follow the manufacturer's instructions for reconstitution and use. The buffer can be refrigerated.

6.2.4 CHROMagar O157

6.2.5 Blood Agar Plates (BAP)

6.3 Controls

6.3.1 List of Controls

6.3.1.1 Positive Media Control: *E. coli* O157:H7 strain ATCC 43890-GFP from Peter Feng, FDA

6.3.1.2 Negative Media Control: *E. coli*

6.3.2 The positive control, *E. coli* O157:H7 (ATCC 43890-GFP) should appear mauve on CHROMagar plate, while the negative control *E. coli* should appear blue.

6.4 IMS analysis

The following steps are based on the manufacturer's instructions for manual IMS described in the package insert. To avoid cross contamination careful pipetting is essential.

6.4.1 Slide out the removable magnetic plate and load the necessary number of 1.5 mL microcentrifuge tubes into the Dynal MPC-S rack.

6.4.2 Resuspend the Dynal anti-*E. coli* O157 Dynabeads by gently shaking or using vortex mixer. Add 20 µL Dynabeads to each tube in the rack.

6.4.3 Add 1.0 mL mEC+n overnight culture that tested positive for *E. coli* O157:H7 by BAX PCR to above tubes. Close the caps tightly.

Note: Avoid cross contamination by carefully pipetting and not touching the tubes. Opening and closing one tube at a time during processing is recommended.

6.4.4 Invert the rack a few times to mix the contents and incubate for 10 minutes at room temperature with gentle shaking.

Note: Gentle and constant mixing will help to capture more bacteria and this can be done by rotational or horizontal mixers.

6.4.5 Insert the magnetic plate and gently invert several times to allow the beads to concentrate on the sides of the tubes. Let the tubes stand for 3 minutes undisturbed.

6.4.6 Open the tubes (use cap opener), gently aspirate supernatant and the remaining liquid in the tube's cap and discard.

6.4.7 Remove the magnetic plate from the Dynal MPC-S and add 1.0 mL wash buffer. To avoid contamination, do not touch the tubes with the pipette.

6.4.8 Close the caps and gently invert the Dynal MPC-S a few times to resuspend the beads.

6.4.9 Repeat steps 6.4.5 through 6.4.8.

6.4.10 Repeat steps 6.4.5 and 6.4.6.

6.4.11 Remove the magnetic plate and add 100 µl of wash buffer. Mix gently in a vortex mixer.

6.5 Isolation of *E. coli* O157

6.5.1 Dispense 50 µL of the enriched sample to each of two CHROMagar O157 plates. Using a sterile swab or a hockey stick, spread the sample over the half of the plate. Using a sterile loop, streak back and forth between a quadrant of swabbed area and a quadrant of unstreaked area several times.

6.5.2 Incubate the plates at 35±2°C for 18-24 hours. Observe plates for *E. coli* O157 colonies which should appear mauve while other *E. coli* colonies should appear blue

6.6 Confirmation of *E. coli* O157

6.6.1 Pick up to three mauve colonies, subculture to BAP, and identify at least one colony using VITEK. If colony does not identify as *E. coli* O157, repeat VITEK on other isolates.

6.6.2 Refer to SOP MDP-SHIP–03 for preparation of organism for shipment.

6.7 Reporting

Data shall be reported according to SOP MDP-DATA-01.

7. Safety:

E. coli O157:H7 is a human pathogen and is known to cause disease with a low infectious dose. The laboratory personnel must follow CDC guidelines for working with Class II pathogens. Use of lab coats, gloves, and eye protection is mandatory. A Class II biosafety laminar flow hood (cabinet) is recommended.

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- Established procedures for IMS and cultural confirmation of *E. coli* O157